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Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852

Subject: Docket No. 98D–1195
Draft Guidance for Industry on Bioanalytical Methods Validation for Human Studies
(Federal Register: January 5, 1999 [Volume 64, (63 FR 517])

Genentech, Inc. would like to commend the Food and Drug Administration (FDA) in its efforts to provide guidance to industry in establishing bioanalytical methods validation for human studies. However, we would like the agency to clarify the scope of the application of this draft guidance. Specifically, does the guidance in this document apply to both recombinant proteins and small molecule drug development or is the scope limited to small molecules only?

For companies like Genentech that have well characterized recombinant protein therapeutics approved by both the Center for Drug Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER), it would be advantageous to have this guidance reflect both Center's expectations and current thinking. Guidance on bioanalytical methods validation for human studies is extremely valuable to industry and should be consistent between both Centers. In addition, we recommend that the guidance be updated to reflect the *ICH-Q2A Text on Validation of Analytical Procedures* (March 1995) and *ICH-Q2B Validation of Analytical Procedures: Methodology* (November, 1996).

Genentech, Inc. understands and appreciates the Center's hard work in putting together a guidance of this type, however, we suggest that the draft guidance be modified to include:

- clear definition and scope of drug products covered by the guidance (e.g. recombinant therapeutics),

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- identification and guidance of other bioanalytical methods in addition to the two that are listed, [chromatography and High-Pressure Liquid Chromotography(HPLC)],
- both CBER and CDER perspectives on bioanalytical methods validation for human studies, if it is to be applied to both protein and small molecules, and
- harmonization with ICH-Q2A and Q2B.

In the event that this guidance will cover recombinant protein therapeutics, we have provided specific comments for identified sections of the guidance document (Attachment 1). If you have any questions regarding this information, please contact Taylor Burtis, Regulatory Affairs – Policy at (650) 225-7729.

Sincerely,

Robert L. Garnick, Ph.D.
Vice President
Regulatory Affairs

cc: Rebecca Devine, Ph.D.
Center for Biologics Evaluation and Research (HFM-10)

Attachment 1

Draft Guidance for Industry: Bioanalytical Methods Validation for Human Studies

Issue / Topic	Comments/ Recommendations
I. INTRODUCTION p-1	
	• The introduction seems to address all bioanalytical methods used in clinical, but not preclinical, studies. Genentech suggests that the types of assays covered and types of studies to which these guidelines apply be more precisely defined. • The second paragraph states that "this guidance should also apply to other analytical techniques...." The guidelines seem to be derived mainly from chromatographic analytical methodology and do not seem appropriate for all analytical technologies. Please expand on "analytical technics" e.g. immunoassays, bioassays, nucleic acid technology, enzymatic assays, and morphology/pathology.
II. BACKGROUND p-1	
	• In the third paragraph of this section it states "when changes are made to a previously validated method, the analyst should exercise judgment as to how much additional validation is needed." The paragraph then goes on to describe examples of major and minor changes that would require re-validation. Genentech recommends that a different approach be taken to identify prospectively the criteria for revalidation. We recommend that as part of the original validation, based on the identified critical parameters, the criteria for revalidation and the scope of revalidation be defined. We feel this will lead to a more consistent assay method application. • Please clarify what is meant by clause in sentence number four of the 3^{d} paragraph "The number of extractions of the biological matrix" • In the fourth paragraph of this section it states, "The analytical laboratory conducting BA and BE studies should closely adhere to FDA's Good Laboratory Practices (GLPs) (21 CFR Part 58) and to sound principles of quality assurance throughout the testing process." Genentech agrees with the Agency's position but recommends that the Agency recognize in the guidance document that there will be some analytical assays that will not comply with GLPs based on the nature of the assay e.g. genetic assays.

III. REFERENCE STANDARD p-2	
	• In Genentech's experience with clinical and preclinical studies, the important issue is not always comparison to a master standard as described in this section of the guidance document, but the equivalency of the reference standard to the material used in the clinical study. Genentech recommends that Agency include this concept. • This section list three types of reference standards: (1) certified reference standards (e.g., USP compendial standards); (2) commercially supplied reference standards obtained from a reputable commercial source; and/or (3) other materials of documented purity custom-synthesized by an analytical laboratory or other noncommercial establishment. Genentech recommends that a fourth type of reference method be added to the list – a specified manufactured lot of the drug product. This would allow a company to prepare its own standard for a proprietary unique compound. • This section should be harmonized with Section 2.11. Reference Standard of the ICH Q6A *Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical* and Section 2.2.1 Reference standards and reference materials of the ICH Q6B *Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products* guidance documents.

IV. PRE-STUDY VALIDATION p-3	
A. Specificity p-3	

	• The first sentence of this section defines specificity as "...the ability of an analytical method to differentiate and quantitate the analyte in the presence of other constituents in the sample and refers directly to the ability of the method to produce a response fora single analyte (Karnes 1991)."
	  • This definition, if applied to protein drugs measured by ELISA or bioassay, would often be impossible to accomplish and is of questionable appropriateness. Protein drugs often undergo some form of metabolism in vivo and those metabolites may still convey substantial biological effect. This issue is a matter of considerable literature debate; we recommend that the Agency revisit its definition of "Specificity" and expand it to reflect the nature of protein therapeutics.
	  • Please clarify the term "single analyte" used in this definition. Complex recombinant proteins often exist as variants, both at the oligosaccharide and amino acid level. Any drug, small or large, may have chiral centers. Should every assay run on every sample be able to discriminate between enantiomers?
	  • This definition of "single analyte is extremely restrictive and fails to recognize the current multi-analyte capable methods e.g. Liquid Chromotography – Mass Spectromity/Mass Spec (LC-MS/MS), multiplex immunoassays, and multiplex gene assays.
	
	• In biological and immunological assays, it is often important to measure the endogenous concentration of a biological agent. The second paragraph of this section needs to be expanded to include provisions for this possibility.
	
	• It is not always advisable to change the analytical method to "eliminate the interference" as stated in first paragraph of this section. Substances that "interfere" with the analysis of a substance may be important to understanding the metabolism and distribution of the drug. Genentech recommends that the Agency reassess its position.
	
	• The last paragraph of this section states, "Potential interference from nicotine and common OTC drugs and metabolites, such as caffeine, aspirin, acetaminophen, and ibuprofen should be routinely tested." This is reasonable for methods that lack strong selectivity such as LC-UV. However, methods based on high selectivity such as ELISAs and LC-MS/MS would not be expected to suffer from these types of interferences. This effort seems excessive for methods with inherent high selectivity. Would the Agency please clarify its position?

B. Calibration Curve p-4	

	• The first paragraph, fourth sentence of this section states "A calibration curve should be prepared in the same biological matrix as the samples in the intended study by spiking with known concentrations of the analyte." Genentech does not agree with this statement for the following reasons:
	  • The calibration curve should not be prepared in the same biological matrix as the samples if the objective is to measure endogenous concentrations of the analyte.
	  • If endogenous analyte is present, the calibration curve should be prepared in a defined "assay buffer"
	  • When the biological matrix and assay buffer are equivalent, quantification of the analyte in the biological matrix should be compared to quantization in assay buffer.
	• We would like the Agency to rewrite this section to include endogenous analytes.
	
	• The sixth sentence of the first paragraph of this section states, "The number of standards used in constructing a calibration curve will be a function of the anticipated range of analytical values and the nature of the analyte/response relationship. Concentrations of standards should be chosen on the basis of the concentration range expected in a particular study." Genentech feels that this may not be possible or even practical. For example; an IV bolus of drug may generate one or two very high concentration points and then trail off to low levels. If the analytical method is crafted to focus on the low levels, a very broad standard range may compromise the quality of the low end. Dilution of highly concentrated samples is routinely done and should be allowed.
	
	• Genentech recommends that the factors for a calibration curve should be dependent on the assay method used and not "blanket" criteria specifications.

1. LOQ	

	The first bullet point in this section states "No interference present in blanks at the retention time of the analyte at this concentration, or typical response at this concentration at least five times greater than any interference in blanks at the retention time of the analyte"
	  • "No interference" does not appear to be consistent with the allowance for interference in "10% of blank samples" referenced in the 2nd paragraph of Section A. Specificity. Please clarify.
	
	  • "at least five times greater" is very tight limit. Can the Agency provide a reference demonstrating why this is required?

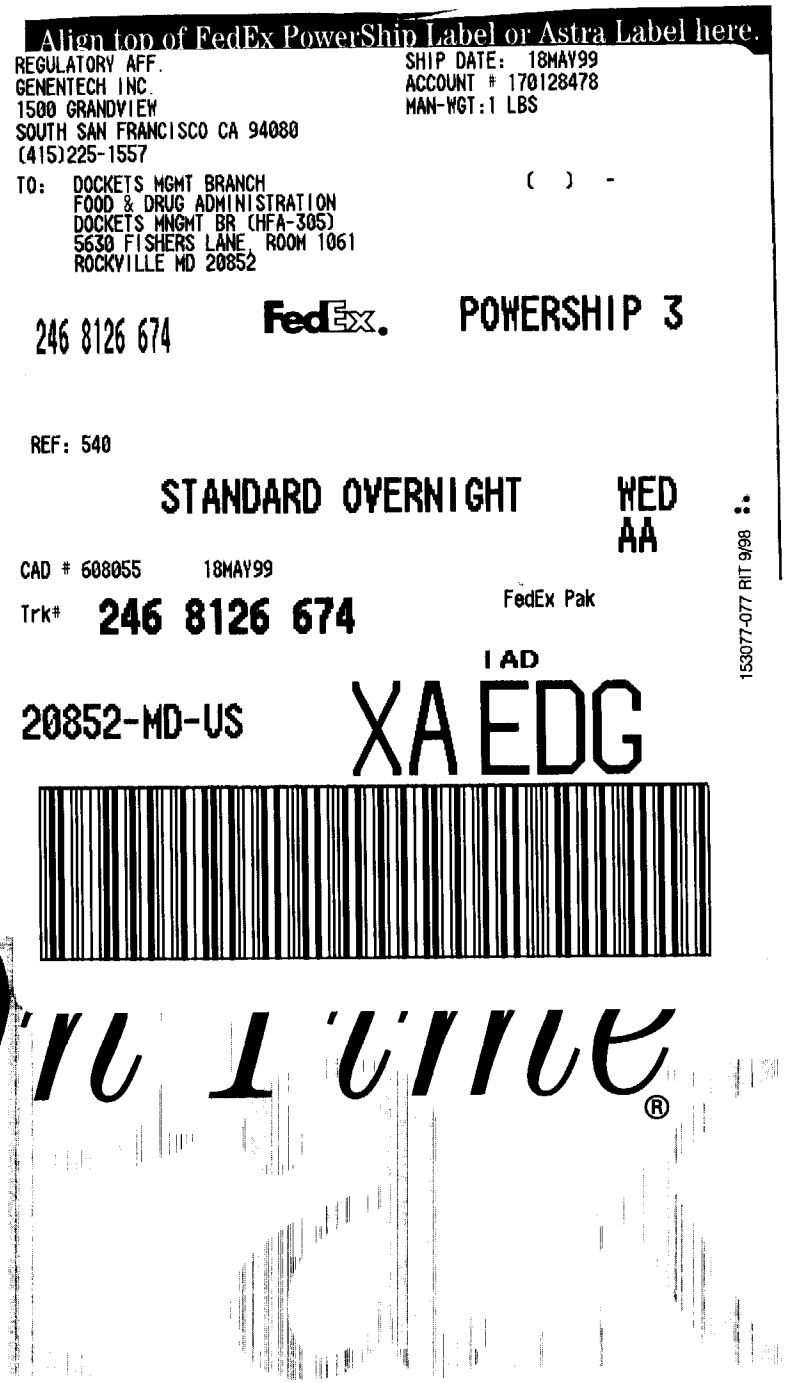
2. Linearity	
	• The first sentence of this section assumes that only linear methods will be used. Genentech recommends that the Agency reconsider its position and allow weighting for non-linear methods e.g. ELISA and bioassays, as well as for linear methods like HPLC.
C. Precision, Accuracy, and Recovery p-5	
	• The recommendations for precision, accuracy, and recovery seem to be specific for chromatographic assays. Genentech recommends this section be expanded to include immuno- and bio-assays. • Sentence three of the section defining "Recovery states, "Although recoveries close to 100% are desirable, the extent of recovery of an analyte and/or the internal standard may be as low as 50 to 60% if the recovery is precise, accurate, and reproducible. Recovery experiments should be performed by comparing the analytical results for extracted samples at three concentrations (low, medium, and high) with unextracted standards that represent 100% recovery." Genentech does not agree with the "50 to 60%" requirement. We recommend that recovery should be required to be consistent (within some predetermined level of variation) across a wide range of concentrations. Furthermore, there may be situations where 50-60% is impossible. For example, if a (small molecule) method developer doing LC-MS/MS set the mass spec to uni-mass resolution and was measuring a small molecule which included three chlorines, the very best that could be recovered is about 33% of the total compound in the sample.
D. Quality Control Samples p-6	
	• Genentech does not agree with the agency's four types of quality control samples. We recommend three levels of controls. Genentech questions the value of the suggested "LOQ QC sample since, in theory, 50% of the time it will not produce a quantifiable result. The percent accuracy should include a provision to do the calculation without the "nominal concentration". Instead, it should be possible to use the measured concentration of an identical spike into "assay buffer" in the absence of biological matrix. • Please provide definitions and examples for: a blank sample, matrix blank and reference standard.

E. Stability p-7	
1. Freeze Thaw Stability	

	• Genentech would like the agency to redraft this section taking into consideration the following:
	  • The guideline specifically calls out thawing without assistance; this is not general practice and dose not reflects what happens to product. It is common practice that thawing happens by heat or freezing is done quick/flash.
	  • The guidance states "Testing for freeze and thaw analyte stability should be determined during three freeze and thaw cycles." It's Genentech's opinion that the establishment of the number of how many freezes and thaws cycles should done to demonstrate stability should be based on a scientific assessment and not an arbitrary number. Genentech recommends that "three freeze and thaw cycles" should be changed to "a relevant number of freeze and thaw cycles.

5. Autosampler Stability	• Genentech recommends that this section should be expanded to include instrumentation and analytical methods in addition to HPLC.
F. Acceptance Criteria p-9	
	• Genentech would like to have this section reflect the current ICH–Q2B Validation of Analytical Procedures: Methodology.
V. IN-STUDY VALIDATION P-9	
	• We recommend that this section of the guidance be expanded to include validation for linearity of dilution and sample re-assay. • It is unclear in this section how the following terms are being applied; calibration curve, spiked sample, and unknown sample. Several of our readers thought the author was using them interchangeably. Please clarify. These terms have been defined in ICH- Q2A and Q2B. • Genentech strongly disagrees with the last line of the first paragraph of this section "All study samples should be analyzed in a single run." Genentech is interested in why the Agency has taken this position. Some clinical trials may last for many months or even years. It would not be scientifically sound or practical to store and perform single patient runs. Stability of the analyte would be a major issue. In addition, the validation of the assay will have demonstrated its precision, accuracy, robustness and reproducibility.

VI. DOCUMENTATION p-10	
	The information that is requested to be sent in as part of a submission appears to be overly burdensome and excessive for the following reasons: • It should not be necessary to submit calibration curves and in-study validation data, since these are all included in the study records. • It should not be necessary to submit "complete serial chromatograms". This information is available upon study audit. • It should not be necessary to submit all raw data, calculations and re-assay sets, since the prospective procedures for these are in the pre-study validation. Genentech would like CDER to work with CBER to identify uniform documentation needs for support of bioanalytical assay validation in a IND, BLA and NDA submission.



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